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## Separability of racemates in chiral chromatography

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# Summary

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In recent years, the impact of chirality in the design, development and utilization of drugs has gained widespread recognition. As a result, there has been a dramatically increasing demand for chiral separations, *i.e.* methods that can separate and/or distinguish individual enantiomers. Among these methods, high-performance liquid chromatography (HPLC) plays a pivotal role.

When an analyst faces the task to develop a separation method for a given problem, it is important that there exists sufficient background knowledge on the potentials of the techniques available in relation to the analyte(s) of interest, so that a rational choice can be made towards the best possible solution. For the HPLC analysis of non-chiral analytes, extensive theoretical knowledge is available. Yet, despite various efforts, when it comes to the HPLC of chiral analytes, knowledge about the basic principles is still fragmentary and most of the phase systems available are non-predictable with regard to their enantioselectivity and retention properties for a given chiral problem. Thus, the choices made are usually a matter of trial and error.

With the studies described in this thesis, we intended to make the retention and/or separation behaviour of pharmaceutically relevant racemic compounds on different chiral stationary phases more understandable, if not more predictable. To us, investigating the chromatographic behaviour of structurally related analogues on various chiral stationary phases (CSPs) and coupling these experimental results to molecular modeling studies of the analytes and the respective stationary phases appeared the key to unravel the mechanisms leading to chiral discrimination.

In **Chapter 1**, the subject 'chirality' is introduced in general terms. The different types of stereoisomerism are described and the consequences of chirality in living systems are being addressed with special emphasis on the pharmacodynamics and pharmacokinetics of drugs. Also, the guidelines for the approval of newly developed drugs are mentioned. The growing importance of chirality in the pharmaceutical and biomedical fields is reflected in the ever increasing demand for separation techniques of chiral compounds in all kinds of matrices. Methods for the resolution of optical isomers are reviewed. In doing so, the main interest is directed towards analytical methods, and then especially towards HPLC. The most common types of CSPs are described in more detail and newer strategies to achieve enantioseparations are introduced. Finally, three series of structurally related compounds, which were used for the structure-retention relationship studies in this thesis, are presented. A rather large number of structurally related racemic 2-amidotetralins became our main focus. The other series consisted of mianserin and naproxen analogues, respectively.

In **Chapter 2**, the results of the chromatographic investigations on the polysaccharide stationary phases Chiralcel OD and Chiralpak AD, being based on the tris(3,5-dimethylphenylcarbamate)s of

cellulose and amylose, respectively, are presented and discussed. Generally, the two CSPs exhibit a complementary character for the separation of a wide variety of racemic analytes. This means that racemates that separated well on one phase were separated less on the other and vice versa. Overall, for the 2-amidotetralins, the cellulose-based phase showed a better selectivity than the amylose-based phase. As far as mianserin and its 8-nitro and 8-triflate analogues are concerned, on both polysaccharide CSPs good to excellent enantioselectivities were observed, whereas the 8-hydroxy analogue was hardly resolved on either column. Furthermore, the analyses on the two stationary phases revealed a dependency of the retention of the first eluted enantiomers on the lipophilicity of the analytes, expressed as calculated octanol-water partition coefficients  $\log P$ . For the 6-azamianserin analogues, comparable enantioselectivities were obtained on the two columns, however, these were generally lower than those obtained for the mianserin analogues. Moreover, the relation between the retention of the first eluted enantiomers and the lipophilicity parameter was observed for the Chiralcel OD column only, whereas this was not true for the Chiralpak AD column. For pharmacological profiling, the enantiomers of the 8-triflate analogues of mianserin and 6-azamianserin were resolved by means of HPLC on a semi-preparative Chiralcel OD column.

The results of the HPLC and supercritical fluid chromatography (SFC) analyses of naproxen-derived analogues and racemic 2-amidotetralins on various chiral brush-type phases, which were mainly based on the 4-(3,5-dinitrobenzamido)-3-allyl-1,2,3,4-tetrahydrophenanthrene selector, are presented and discussed in **Chapter 3**. The  $\pi$ -aromaticity of naproxen, *i.e.* 6-methoxy- $\alpha$ -methyl-2-naphthaleneacetic acid, was altered by the substitution of the methoxy group with a hydroxy group on the one hand, and with the electron-withdrawing methylsulfonyloxy (mesylate) and trifluoromethylsulfonyloxy (triflate) groups, respectively, on the other. To a certain extent, the  $\pi$ -acid/ $\pi$ -base interaction, which is supposed to be a major principle in the chiral recognition on the Whelk-O phase, could be confirmed in that the enantioselectivities for the methoxy and hydroxy analogues were higher than those for the mesylate and triflate analogues. Yet, there was no gradual change in enantioselectivity between all four analogues based on their increasing  $\pi$ -acidity. Remarkably, the triflate analogue showed good enantioselectivities at rather short retention times. This unique combination indicates that the triflate group may become useful for the development of new chiral selectors. The analysis of 2-amidotetralins on different brush-type CSPs by means of HPLC and SFC revealed that the *cis*-(3*R*,4*S*)-Whelk-O 1 phase was most suitable among the CSPs chosen for the resolution of these analytes. The lower enantioselectivities obtained on the latter phase as compared to the Chiralcel OD column were attributed to the structural features of the 2-amidotetralin skeleton, which would prevent this class of compounds from simultaneously interacting with the chiral recognition sites of the Whelk-O selector. In most of the cases, decreasing the temperature resulted in improved enantioselectivities, going along with increasing retention times. In the course of the study, a number of novel chiral selector compounds were synthesized. The chromatographic evaluation of those selectors was done on the Whelk-O 1 phase and a (*S*)-naproxen-derived CSP. It revealed the clear potential of the 3,5-ditriflatebenzamido modified Whelk-O 1 selector, as well as of 2,3-dihydro-1-(3,5-dinitrobenzamido)-(8-methoxy)-naphtho[2,1-*b*]pyran as novel chiral selectors.

In **Chapter 4**, current strategies to explain and/or predict the interactions between enantiomers and chiral selectors are summarized. Moreover, a model for the visualization of the possible

stereoselective interactions between selected 2-amidotetralins and the Whelk-O selector is presented. Hydrogen bonding and charge-transfer interactions appeared to be most important for a successful enantioseparation. The elution order of one of the selected 2-amidotetralins was predicted by means of the model derived. However, this prediction could not be confirmed experimentally due to the non-availability of the individual enantiomers. The validity of the model generated has to be evaluated further, with other structurally related, as well as non-related analogues.

In **Chapter 5**, concluding remarks on the basis of the studies carried out and some perspectives are given. Although the approach to investigate the retention and separation behaviour of a large series of structurally related racemates did not enable us to derive the spatial arrangement of the interaction sites of the polysaccharide phases Chiralcel OD and Chiralpak AD, it was useful for the Whelk-O 1 stationary phase. On the basis of the experimental data obtained on the latter phase and of proposed chiral recognition mechanisms from the literature, the stereoselective interactions between the Whelk-O selector and several 2-amidotetralins were modelled, which allowed us to (partially) unravel the chiral discrimination process on this stationary phase. Finally, the further investigation of triflate-substituted benzamides as potential chiral selectors was outlined. The combination of good enantioselectivities and short retention times, caused by the substitution with a triflate group, may be advantageous for the development of new chiral stationary phases.